Bone Marrow Transplantation for Adult Sickle Cell Patients

Uzoamaka Nwokorie[1,2] and Nicholas A Kerna[3]*

[1]*University of Science, Arts and Technology, BWI*
[2]*University of Health and Humanities, BVI*
[3]*SMC–Medical Research, Thailand*

***Corresponding Author:** Nicholas A Kerna, POB47 Phatphong, Suriwongse Road, Bangkok, Thailand 10500.
Contact: medpublab+drkerna@gmail.com.

Abstract

Sickle cell disease (SCD) is a common health problem in the United States involving multiple organ damage. Currently, bone marrow transplantation (BMT) is the only curative therapy. Until recently, BMT has not been considered for adults with SCD because of their inability to tolerate the toxicity of intensive conditioning regimens as a result of long-term organ damage from SCD. A new conditioning regimen with much-reduced toxicity has recently been reported with great success in BMT for adult sickle cell patients. However, the reduced-intensity conditioning regimen in adult sickle cell patients has been performed on a small scale. This paper reviews the clinical trials involving the reduced-intensity conditioning regimen for adults with SCD and the approaches to overcome the barriers to the more widespread use of this new method of BMT for adults with SCD. Thus far, the only published clinical trial involving reduced-intensity conditioning for adult sickle cell patients, with survival outcome, reported overall survival of 90% at 30 months post-BMT; however, this study was limited to ten patients. The study suggests that the low toxicity of reduced-intensity conditioning allows for a stable, mixed donor-recipient chimerism and reverses the sickle cell disease phenotype without serious adverse effects.

***Keywords:** Anemia; Bone Marrow; Chimerism; Graft; Sickle Cell Disease; Transplant*

Abbreviations

ACS: Acute Chest Syndrome; allo-SCT: Allogeneic Stem Cell Transplantation; ATG: Antithymocyte Globulin; BMT: Bone Marrow Transplant; CB: Cord Blood; G-CSF: Granulocyte-Colony Stimulating Factor; GvHD: Graft-Versus-Host Disease; GvL: Graft-Versus-Leukemia; HbF: Fetal Hemoglobin; HbS: Homozygous Sickle Hemoglobin S; Hgb S: Hemoglobin S; HLA: Human Lymphocyte Antigen; HSC: Hematopoietic Stem Cell; HSCT: Hematopoietic Stem Cell Transplantation; MHC: Major Histocompatibility Complex; PBSC: Peripheral Blood Stem Cell; RBC: Red Blood Cell; RIC: Reduced-Intensity Conditioning; SCD: Sickle Cell Disease

Introduction

Sickle cell disease (SCD) is a common congenital disorder with multiple organ damage in affected individuals [1]. A single nucleotide substitution at the 6th position of the beta-globin chain in which glutamic acid is replaced by valine, has been established as the cause of hemoglobin polymerization, resulting in sickle-shaped red blood cells (RBCs) [2]. This RBC shape distortion results in chronic anemia and increased hemolytic and vaso-occlusive complications that affect multiple organs.

Polymerization of the mutated hemoglobin molecule under hypoxic conditions causes RBCs to have a sickle-shaped deformity, become dehydrated and rigid, and adhere to vascular endothelium [2]. Anemia, growth delay, frequent infection with encapsulated bacteria, bone infarctions, and chronic organ damage involving the brain, heart, lungs, joints, gallbladder, kidneys, and retina are common complications of SCD, and account for most of the morbidity and mortality [3]. Median life expectancy is estimated to be 42 years for men and 48 years for women with homozygous sickle hemoglobin S (HbS) [3]. Organ damage may occur at an early age, contributing to a mortality of 15% by 18 years [2,3]. The sickle cell gene for HbS is the most common inherited blood condition in the United States. About 70,000 - 100,000 Americans—mostly African Americans—have SCD. About 2 million Americans carry the sickle cell trait [3].

Historical perspective

In 1910, SCD was first described in a patient who presented with pulmonary symptoms [4]. Herrick (1910) coined the term "sickle-shaped" to describe the peculiar appearance of the RBCs of this patient. However, given the patient's symptoms, he was not sure at the time whether the blood condition was a disease by itself or a manifestation of another disease [4]. After several years, similar cases were described, supporting the idea that this was a new disease entity and providing sufficient evidence for a preliminary clinical and pathological description [5]. Shortly thereafter, Hahn and Gillespie (1927) suggested that anoxia caused RBC sickling by demonstrating that shape changes could be induced by saturating a cell suspension with carbon dioxide [6]. Scriver and Waugh (1930) proved this concept *in vivo* by inducing venous stasis in a finger using a rubber band. They showed that stasis-induced hypoxia dramatically increased the proportion of sickle-shaped cells from approximately 15% to more than 95% [6]. These seminal studies were noted by Linus Pauling, who was the first researcher to hypothesize in 1945 that the disease might originate from an abnormality in the hemoglobin molecule [1]. This hypothesis was validated in 1949 by the demonstration of the differential migration of sickle versus normal hemoglobin as assessed by gel electrophoresis [1]. That same year, the autosomal recessive inheritance of the disease was explained [1]. Around the same time, Watson *et al*. (1948) predicted the importance of fetal hemoglobin (HbF) by suggesting that its presence could explain the more extended period necessary for sickling of newborn RBCs compared with those from mothers who had "sicklemia" [7]. Ingram (1958) demonstrated that the mutant HbS differed from normal hemoglobin by a single amino acid [8]. This finding was followed by studies that analyzed the structure and physical properties of hemoglobin S (Hgb S), which formed intracellular polymers upon deoxygenating [8]. These studies placed SCD at the leading edge of investigations to elucidate the molecular basis of human diseases. Abnormal hemoglobin polymerization upon deoxygenating gave rise to the idea of a hematopoietic stem cell-based approach as a therapeutic option for SCD [2].

In 1984, the first successful BMT for SCD was reported [9]; in 1996, the first large-scale study of 22 children with SCD was published [10]. The participants, all less than 16 years old, received BMT from human lymphocyte antigen (HLA)-matched sibling donors. BMT was performed after receiving busulfan, cyclophosphamide and antithymocyte globulin (ATG), or alemtuzumab-based regimens. The criteria for participation included a history of stroke, recurrent acute chest syndrome, abnormal brain imaging, retinopathy, or bone disease. Evaluation of two years post-BMT indicated that 99% were alive, 72% had stable chimerism, and 18% had graft rejection [10]. However, neurologic events, including seizures and cerebral hemorrhage, were reported in seven patients (31%) [10]. In Belgium, the first 50 patients with SCD—who received an HLA-matched sibling BMT using bone marrow (48 patients) or cord blood (2 patients), after conditioning with busulfan and cyclophosphamide-based regimens—resulted in 80% having stable chimerism [11].

The evidence that stable mixed hematopoietic chimerism can result from myeloablative BMT in patients with SCD [10,11] led to the consideration that this application could be an effective treatment for SCD, with substantially reduced transplant-related morbidity and mortality [12]. Some researchers suggested that small amounts of donor bone marrow chimerism may result in high levels of normal hemoglobin, as sickled RBCs have a survival disadvantage [12]. In a report by Wu *et al*. (2007), patients with SCD developed stable but partial donor myeloid chimerism after myeloablative BMT [12]. Fractions of HbS in blood were 0%, 0%, and 7% in the three patients whose donors had a normal Hb genotype, which corresponded to a donor myeloid chimerism level of 67%, 75%, and 20%, respectively.

None of the patients experienced painful events or other clinical complications of SCD after BMT [12]. Wu *et al*. (2007) concluded that a significant reduction in morbidity and mortality from SCD might occur with low levels of donor myeloid chimerism [12]. Given the results of this study and the high toxicity of myeloablative conditioning, there is a growing interest in a nonmyeloablative procedure that can produce stable mixed chimerism [13]. With the foundation in research reported above, the following discussion will explore BMT as a therapeutic option for SCD.

Discussion

BMT from a nonaffected donor, usually an HLA-matched sibling, is the only known curative therapy for SCD [14]. The lack of an HLA-matched sibling has been the most frequently reported drawback of this procedure [15]. Nationwide, about 200 children have been reported cured of SCD after undergoing a myeloablative conditioning regimen [16]. Until recently, BMT has not been considered for adults with severe SCD because of their perceived inability to tolerate myeloablative conditioning regimens [17]. The development of less toxic, nonmyeloablative, or reduced-intensity conditioning (RIC) regimens has long been proposed [17]. Its successful application in BMT for adults with SCD has now been reported [18].

Sources of allogeneic hematopoietic stem cells

For many years, bone marrow aspirated from the iliac crest was the primary source of hematopoietic stem cells for transplantation [15]. In autologous transplantation, peripheral blood stem cells (PBSCs) replaced aspirated bone marrow during the 1990s. During the last decade, a similar trend has been seen in allogeneic stem cell transplantation (allo-SCT) for patients with hematological malignancies [19]. After 5–6 days of injections of granulocyte colony-stimulating factor (G-CSF), a high number of stem cells can be harvested from the blood by aphaeresis [20]. The PBSC graft has a higher number of nucleated cells, CD34- and CD3-positive cells, as well as natural killer cells (compared with the bone marrow graft) and results in faster engraftment of neutrophils and platelets [20]. Despite the high number of T-cells, the incidence of acute graft-versus-host disease (GvHD) is similar to that after transplantation of bone marrow; likely, in part, due to a shift from Th-1 to Th-2 cells. However, chronic GvHD is more common and, therefore, PBSC is less often used for patients with nonmalignant disorders (such as SCD) due to a lack of the benefit from the graft-versus-leukemia (GvL) effect correlated with chronic GVHD [20]. The use of granulocyte-colony stimulating factor (G-CSF) to mobilize stem cells from a donor's bone marrow into the peripheral circulation demonstrated rapid engraftment and a low incidence of GvHD [21]. This finding led to further studies to determine the feasibility of using PBSC for patients with SCD [11,21,22].

Another source of allogeneic hematopoietic stem cells is the cord blood (CB). CB, usually wasted during delivery, is rich in hematopoietic stem cells and can be used as a stem cell source for allo-SCT [16,23]. In many countries, banks of cryopreserved CB units are established and >100 000 units are available for transplantation and >4200 transplants with CB grafts have been performed [23]. The advantages of CB are the rapid availability and the reduced risk for GvHD due to the relative lack of mature T cells [16]. Thus, some degree of mismatch can be allowed. However, drawbacks are a higher incidence of graft failure and slower engraftment resulting in more infections [23]. The number of nucleated cells and CD34 cells per kilogram bodyweight of the recipient is approximately one log lower compared with bone marrow as a stem cell source. Several studies have shown the importance of a high CB cell dose and more than 2×10^7 nucleated cells/kg is recommended. This finding limits the use of CB for adult patients [23]. However, various ways have been explored to increase the cell number: the pooling of two units or *in vitro* expansion being most explored. Although the *in vitro* expansion is theoretically appealing, the clinical breakthrough has yet to occur. Nevertheless, the pooling of two units has been performed with promising results [16,23].

Currently, allo-SCT for adult patients with SCD utilizes related or identical donors [18]. This limitation is a significant barrier to allo-SCT for sickle cell patients, given that only a few have healthy compatible siblings [18]. Matthew *et al*. (2009), reported that out of 59 patients that were HLA-typed only 13 had compatible siblings [18].

Conditioning regimens

The chemotherapy or irradiation, given immediately before a transplant, is called the conditioning or preparative regimen. The purpose of the conditioning regimen is to help eradicate the patient's disease before the infusion of hematopoietic stem cells (HSCs) and to suppress immune reactions [13]. In myeloablative conditioning, a combination of cyclophosphamide with busulfan or total body irradiation is commonly employed [24-26].

Allogeneic hematopoietic stem cell transplantation (HSCT), using conventional standard myeloablative conditioning regimens, is well established as a treatment option [24]. However, with extensive short- and long-term morbidity and a high mortality rate associated with this procedure, it is not recommended for many high-risk patients with nonmalignant diseases, such as adult sickle cell patients [17,26]. This limitation led to an increased interest in developing a less toxic conditioning regimen or nonmyeloablative conditioning [27,28].

Nonmyeloablative conditioning or RIC uses lower doses of chemotherapy and radiation, which are too low to eradicate all of the bone marrow cells of a recipient [27,28]. The significant advantage of reduced-intensity regimens is that the preparative therapy spares primitive hematopoietic precursors of host origin, such that autologous hematopoietic cells would be expected to recover within one month in the absence of donor stem cell infusion [13]. Most patients treated with these protocols have been selected for their inability to tolerate fully myeloablative conditioning due to various comorbidities [13,27,28].

In patients with nonmalignant diseases, such as sickle cell patients, RIC is used to replace defective host hematopoietic cells with healthy donor cells [19]. Hematopoiesis following RIC changes gradually from host to donor origin [13]. The most widely used RIC protocols with dose-variations include the Seattle protocol: fludarabine, low-dose TBI, and post-transplant CsA and mycophenolate mofetil [29]; the Jerusalem protocol: fludarabine, fresenius antithymocyte globulin, and minimal cytotoxic therapy (either BU 8 mg/kg or CY 120 mg/kg) [13]; and the MD Anderson protocol (and its variations): fludarabine and Ara-C, melphalan, and CY or an anthracycline [28].

Different RIC protocols vary in their intensity and many studies have been done to determine the effects of various regimens [13,19,29,30]. Some studies demonstrated that rapamycin might be more beneficial than cyclosporine in the prophylactic treatment for GvHD, after allo-SCT [30].

Given the results of these studies, there is continuing interest in protocols that are nonmyeloablative and could produce stable mixed chimerism [12]. The problem to overcome is one of tolerance as there must be a coexistence of both host and donor cells for stable mixed chimerism to be achieved. While the initial protocols used relatively nonspecific immunosuppressive agents to induce transplantation tolerance, a recent murine study focused on blocking T-cell activation pathways as a targeted approach for developing donor-specific tolerance [31]. This study demonstrated that the disruption of the T-cell costimulation signal mediated by the CD28/B7 or CD40/CD40L pathways (at the time of BMT) could lead to the anergy of donor-reactive host T cells and produce long-term tolerance [31]. This murine-BMT study—that employed nonmyeloablative preconditioning with low-dose busulfan coupled with costimulation blockade of the CD28/B7 and CD40L pathways—produced long-term, mixed chimerism and robust tolerance to a fully major histocompatibility complex (MHC)-mismatched allograft in the mouse [31].

The successful use of the costimulation blockade to cross allogeneic barriers suggested the feasibility of clinical application for human SCD and other nonmalignant hematologic disorders [31]. In a study involving ten adult sickle cell patients, Mathew et al. (2009) utilized costimulation and a nonmyeloablative conditioning regimen to produce mix chimerism. The conditioning regimen consisted of alemtuzumab and a single dose of 300cGy of total body irradiation, plus oral sirolimus (rapamycin), started a day before the transplantation. Alemtuzumab, an antibody directed against CD52, was used to deplete T cells and B cells, thereby preventing GvHD. The study suggested that rapamycin is better than cyclosporine in maintaining chimerism in the absence of long-term immunosuppression and in preventing GvHD [18].

Conclusion

The first successful bone marrow transplant for sickle cell disease was reported in 1984, establishing the procedure as the only therapeutic option for the disease [14]. Until recently, this procedure was not considered for adult patients. Even in children, the widespread adoption of this treatment option has been slow to develop [18]. There are many impediments to more widespread utilization of bone marrow transplants in sickle cell patients in general. One of the most significant drawbacks is the risk-benefit factor. Patients with sickle cell disease are characterized as high-risk if they have had a subclinical stroke, seizure, recurrent severe acute chest syndrome (ACS), chronic unremitting pain, or early evidence of end-organ damage (such as pulmonary hypertension), which are the screening criteria for bone marrow transplant [14,18]. These risk factors pose a challenge in determining the appropriate timing of a bone marrow transplant, given that organ damage can occur at any age, resulting in up to 15% mortality by 18 years of age [2,3]. Another significant limitation is the lack of compatible donors. Given that only a few sickle cell patients have healthy siblings [18], more studies are urgently needed to explore the use of unrelated donors.

While published reports are few in number and small in size, they appear to demonstrate curative treatment for adult sickle cell patients using the reduced-intensity conditioning approach [18]. The use of a nonmyeloablative conditioning regimen and costimulation before allogeneic stem cell therapy reduces treatment-related toxicity; thus, this approach holds great appeal for the treatment of adult patients with sickle cell disease [31]. The success of the study involving ten adult sickle cell patients was attributed to the use of rapamycin (sirolimus) to prevent graft-versus-host disease [18]. This finding provides a strong rationale for further development of a reliable nonmyeloablative stem cell therapy approach that fosters stable mixed chimerism. Also, it suggests a promising role for gene therapy in sickle cell patients. However, the safety of gene therapy remains a significant challenge.

BMT with HLA-matched sibling donors using RIC is a therapeutic option for patients with SCD. The application of this treatment modality in adult patients with SCD is still in its infancy. Only one study has been published. Longer follow-up and multicenter clinical trials are needed to validate the procedure and guide clinicians. A major barrier to multicenter trials appears to be a lack of HLA-matched sibling donors. Exploration of the use of alternative donors would increase the potential pool of donors and ultimately increase the cure rate for this disease.

Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

References

1. Pauling L., *et al*. "Sickle cell anemia: A molecular disease". *Science* 110.2865 (1949): 543-548. https://www.ncbi.nlm.nih.gov/pubmed/15395398

2. Ferrone FA. "Polymerization and sickle cell disease: a molecular view". *Microcirculation* 11.2 (2004): 115-128. https://www.ncbi.nlm.nih.gov/pubmed/15280087

3. Platt OS., *et al*. "Mortality in Sickle cell, Life expectancy and risk factor for early death". *The New England Journal of Medicine* 330.23 (1994): 1639-1644. https://www.ncbi.nlm.nih.gov/pubmed/7993409

4. Herrick JB. "Peculiar elongated and sickle shaped red blood corpuscles in a case of severe anemia". *Archives of Internal Medicine* 6.5 (1910): 517-521. https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/653371

5. Hanh EV and Gillespie EB. "Sickle cell anemia". *Archives of Internal Medicine* 39.2 (1927): 233-254. https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/535284

6. Scriver JR and Waugh TR. "Studies on a case of sickle cell anemia". *Canadian Medical Association Journal* 23.3 (1930): 375-380. https://www.ncbi.nlm.nih.gov/pubmed/20317973

7. Watson J., *et al*. "The significance of the paucity of sickle cells in newborn Negro infants". *The American Journal of the Medical Sciences* 215.4 (1948): 419-423. https://www.ncbi.nlm.nih.gov/pubmed/18107723

8. Ingram VM. "Abnormal human haemoglobins the comparison of normal human and sickle-cell haemoglobins by fingerprinting". *Biochimica et Biophysica Acta* 28 (1958): 539-545. https://www.sciencedirect.com/science/article/pii/000630025890516X

9. Johnson FL., *et al*. "Bone-marrow transplantation in a patient with sickle-cell anemia". *The New England Journal of Medicine* 311.12 (1984): 780-783. https://www.ncbi.nlm.nih.gov/pubmed/6382010

10. Walter MC., *et al*. "Bone marrow transplantation for sickle cell disease". *The New England Journal of Medicine* 335.6 (1996): 426-428. https://www.ncbi.nlm.nih.gov/pubmed/8663884

11. Vermylen C., *et al*. "Hematopoietic stem cell transplantation for sickle cell anemia: the first 50 patients transplanted in Belgium". *Bone Marrow Transplant* 22.1 (1998): 1-6. https://www.ncbi.nlm.nih.gov/pubmed/9678788

12. Wu CJ., *et al*. "Mixed hematopoietic chimerism for sickle cell disease prevents intravascular haemolysis". *British Journal of Haematology* 139.3 (2007): 504-507. https://www.ncbi.nlm.nih.gov/pubmed/17910640

13. Luznik L., *et al*. "HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide". *Biology of Blood and Marrow Transplantation* 14.6 (2008): 641-650. https://www.ncbi.nlm.nih.gov/pubmed/18489989

14. Walters MC., *et al*. "Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. Multicenter investigation". *Blood* 95.6 (2000): 1918-1924. https://www.ncbi.nlm.nih.gov/pubmed/10706855

15. Panepinto JA., *et al*. "Matched-related donor transplantation for sickle cell disease: report from the Center for International Blood and Transplant Research". *British Journal of Haematology* 137.5 (2007): 479-485. https://www.ncbi.nlm.nih.gov/pubmed/17459050

16. Aamkiewicz TV., *et al*. "Unrelated cord blood transplantation in children with sickle cell disease: review of four-center experience". *Pediatric Transplantation* 11.6 (2007): 641-644. https://www.ncbi.nlm.nih.gov/pubmed/17663687

17. Jacobson DA., *et al*. "Reduced intensity haemopoietic Stem-cell transplantation for treatment of nonmalignant diseases in children". *Lancet* 364.9429 (2004): 156-162. https://www.ncbi.nlm.nih.gov/pubmed/15246728

18. Matthew M., *et al*. "Allogeneic Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease". *The New England Journal of Medicine* 361 (2009): 2309-2317. https://www.nejm.org/doi/full/10.1056/NEJMoa0904971

19. Brodsky RA., *et al*. "Reduced intensity HLAhaploidentical. BMT with post transplantation cyclophosphamide in nonmalignant hematologic diseases". *Biology of Blood and Marrow Transplantation* 42.8 (2008): 523-527. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3086163/

20. Pavletic SZ., *et al*. "Unrelated donor marrow transplant for B cell chronic lymphocytic leukemia after using myeloablative conditioning". *Bone Marrow Transplantation* 30 (2005): 618-625

21. Horan JT., *et al*. "Hematopoietic stem cell transplantation for multiple transfused patients with sickle cell disease and thalassemia after Low-dose total body irradiation, fludarabine, and rabbit antithymocyte globulin". *Bone Marrow Transplantation* 35.2 (2005): 171-177. https://www.ncbi.nlm.nih.gov/pubmed/15531901

22. Van Besien K., *et al*. "Allogeneic stem cell transplantation for sickle cell disease". *Bone Marrow Transplantation* 28 (2001): 545-549. https://www.nature.com/articles/1703208?draft=marketing

23. Locatelli F., *et al*. "Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease". *Blood* 101.6 (2003): 2137-2143. https://www.ncbi.nlm.nih.gov/pubmed/12424197

24. Bernaudin F., *et al*. "Long-term results of related myeloablative Stem-cell transplantation to cure sickle cell disease". *Blood* 110.7 (2007): 2749-2756. https://www.ncbi.nlm.nih.gov/pubmed/17606762

25. Eggleston B., *et al*. "Effect of myeloablative bone marrow transplantation on growth in children with sickle cell anemia: results of the multicenter study of hematopoietic cell transplantation for sickle cell." *British Journal of Haematology* (2007). https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2141.2006.06486.x

26. Bernaudin F., *et al*. "Late effects of myeloablative stem cell transplantation or late effects of sickle cell disease itself". *Blood* 111.3 (2008): 1744. https://www.researchgate.net/publication/242729240_effects_of_sickle_cell_disease_itself_Response_Late_effects_of_myeloablative_stem_cell_transplantation_or_late

27. Chakraverty R., *et al*. "Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a non-myeloablative conditioning regimen". *Blood* 99.3 (2002): 1071-1078. https://www.ncbi.nlm.nih.gov/pubmed/11807015

28. Iannone R., *et al*. "Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia". *Biology of Blood and Marrow Transplantation* 9.8 (2003): 519-528. https://www.ncbi.nlm.nih.gov/pubmed/12931121

29. Horvitz ME., *et al*. "Fludarabine-based nonmyeloablative Stem cell transplantation for sickle cell disease with and without renal failure: Clinical outcome and pharmacokinetics". *Biology of Blood and Marrow Transplantation* 13.12 (2007): 1422-1426. https://www.ncbi.nlm.nih.gov/pubmed/18022571

30. Coemen JJ., *et al*. "Rapamycin, not cyclosporine permits thymic generation and preservation of CD4+CD25+FOxP3+ T cell". *Bone Marrow Transplantation* 39.9 (2007): 537-4523. https://www.ncbi.nlm.nih.gov/pubmed/17351648

31. Jacob Hanna., *et al*. "Treatment of Sickle Cell Anemia Mouse Model With ips cells Generated from Autologous Skin". *Science* 318.5858 (2007): 1920-1923. https://www.ncbi.nlm.nih.gov/pubmed/18063756